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Art. III.—*Further Observations on Onchocerca gibsoni, the Cause of Worm-nodules in Cattle.*

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Since the publication last year by the Commonwealth of Australia of our previous paper on *Onchocerca gibsoni*, several lines of external evidence have become available, bearing on the original home and host, the history of its occurrence and geographical distribution in Australia, and the means of transmission of this parasite, etc., so that it seems desirable that the information should be made public, together with a record of the results of series of experiments undertaken to elucidate the life history.

Historical.

Evidence obtained by J.A.G. during the late expedition to the Northern Territory most strongly corroborates previous statements by others and ourselves that there is a gradually increasing extent of infection by *Onchocerca gibsoni*, the further north the cattle are reared: that is, the further away from the ordinary ports of stock introduction in the south and east. From the information available at the time of their writing (1910, p. 99) Doctors Cleland and Johnston considered that the buffalo, imported into Australia from Timor in 1826-8, was the originating host, though, as shown by ourselves later (1911, pp. 2 and 34), it was at least highly likely that the Timor cattle imported about 1824 and 1840 into the Port Essington settlement were the true original hosts. However, an opportunity was available to J.A.G. of examining a number of buffalo, the descendants of those introduced by Sir Gordon Bremmer in 1824 to Port Dundas, and later to the mainland. These have spread from Port Essington southwards over the swampy plains to very near Port Darwin. These buffalo, so far as his experience goes, are all unaffected with *Onchocerca*, but all the cattle depastured on the same country are more or less affected—indeed, the greatest extent of infection yet seen was in a steer killed at Port Darwin, the region of the brisket showing at least a hundred nodules—so that, although experience is limited as regards the buffalo,

it seems almost certain that had the buffalo imported at the early dates given above been the original and natural hosts, their descendants would be at least as badly affected as the cattle, if not more affected. Were these "worm nodules" at all prevalent in buffaloes, it is certain their presence would be known to some of the buffalo hunters, who invariably remove the briskets along with other parts of the flesh for food. During the past 30 years 100,000 buffalo hides have been exported from Darwin, so that it is unreasonable to suppose that the parasitic nodules would have been entirely overlooked in all these animals. Certainly the Indian Ghi buffalo, imported about 1886 by the South Australian Government to Port Darwin itself, cannot be responsible for the original introduction of this parasite to Australia, for even were these nodules known in them, such nodules had been discovered in Australian cattle long before this. Further enquiries, however, elicited the fact that cattle have been imported from a different source altogether. Captain Everard Home, writing from H.M.S. "North Star," 19th April, 1843, reported to the British Government in a despatch on the Port Essington Settlement, among other things, that there were at that date "1 English cow and a bull, and 2 Indian heifers and 2 cows, . . . besides 6 working oxen and 30 buffaloes." Further, numbers of careful observers among those who hunt buffalo on the Coburg Peninsula are positive that the descendants of these Indian cattle are still there, though, unlike the buffalo, they have not spread across the swampy plains down to the cattle station country. But it seems at least highly probable that they were responsible for the introduction of *Onchocerca*, not only from a comparison of the date of their entry, and that of the discovery of the nodules in Australian cattle, and the wide distribution of the parasite, but also in the light of a well-considered statement by Mr. S. L. Symonds, Government Veterinarian of the Federated Malay States, that the only animal in which he has ever found the *Onchocerca* nodules in those States was an old Indian bullock, the native animals and the buffalo being free.

It must be realised, however, that, if the intermediate host of *Onchocerca gibsoni* be a tick, as some have suggested, or a louse, as we ourselves suggested, and considered very probable from general evidence in our previous paper, since these ecto-parasites can only be conveyed any distance by means of their hosts, the ancestors of the Indian cattle now on the Coburg Peninsula could not be incriminated, for, as already stated, these cattle have never become mixed with the station herds. Assuming, however, some blood-sucking insect such as a biting fly to be the intermediary host, then the possibilities of transference over considerable distances must be admitted. A thorough investigation of the descendants of the Indian and British cattle now on Coburg Peninsula will therefore prove extremely interesting, and

it was a matter of great regret that owing to absence of transport, it was impossible to make such an examination as was intended. It is hoped, however, that at an early date such an opportunity may present itself to test the infection or otherwise of these Indian and British cattle. In view of the statement recently made to us by Dr. de Blieck, Director of the Veterinary Laboratory and Veterinary School at Buitenzorg (Java), that tumours similar to those of *Onchocerca gibsoni* are quite common in Java cattle, it may yet be found that our original inference was correct, viz., that the Timor cattle, introduced some time between 1824 and 1840 into Port Essington, were the responsible agents of introduction.

The importation of Javan native cattle to Port Darwin in 1872 which, as is shown elsewhere, is considered to have been the source of the introduction of the cattle tick (*Boophilus annulatus* var. *microplus*) and of tick fever, may also have introduced the "worm-nodules," yet they could hardly have been the first source of introduction, seeing we have circumstantial evidence of the appearance of these nodules in Queensland at least 40 years ago. It may be noted, however, that the crossbred descendants of those Brahma cattle, when examined, all show more or less *Onchocerca* infection.

General Characters of the Nodules.

In some animals recently examined *post mortem*, the proportion of nodules situated in the deep pectoral muscles was much less than had previously been noted, while in the thigh they were very few in number, and then were situated close alongside the head of the femur. In each case they were more numerous on the right side than on the left. Two cases are here given of two cows from the same district in North Queensland, and kept here under exactly similar conditions (*vide infra*) for 6 and 8 months respectively, B having been killed two months later than A.

	A (5 year old cow).	B (3 year old cow).
Total number of nodules	47 (19L, 28R)	15 (6L, 9R)
Number of nodules in thigh	0	3 (1L, 2R)
Number of nodules containing eggs and living larvae	22	4
Number of nodules containing intraparasitic parasites	10	4
Number of nodules degenerate	15	11

The proportion of nodules in B, containing degenerate parasites, is more typical than in A, and undoubtedly a marked diminution in size of the nodules took place during the months the cows were under constant observation, as determined by frequent manipulation.

We have previously noted the fact that amongst the large number of nodules which we have examined, the female parasite was either in a stage of complete development liberating living larvae, or the nodules contained degenerated parasites; in other words, no immature parasite has ever been found in a nodule. With these two cows for a period of 6 and 8 months there was absolutely no possibility of reinfection. Many of the nodules could be felt under the skin, and as a number of these subcutaneous nodules were found *post mortem* to contain living *Onchocerca* liberating living larvae, it must be assumed that they had been continuously liberating such larvae during the whole period, for as the male lies continuously alongside the female, there is probably continuous fertilisation of the latter.

We have in the previous paper indicated the probability that infection of the bovine takes place during its early years (1st and 2nd) alone. Corroboration of this was obtained by J.A.G. and Dr. Breinl when in the Northern Territory; on one station, the station on which the steer with a hundred nodules had been bred and fattened, an opportunity was obtained of carefully examining two very old bullocks. In one, only one small nodule, and that containing a living parasite, could be discovered; in the other, a few small, circumscribed, thin, circular or oval dense fibrous masses about $\frac{1}{2}$ to $\frac{3}{4}$ inch were found adhering firmly to the muscular fascia. Section showed a calcareous centre. Judging from the invariable infection with living *Onchocerca* of younger cattle on this station, two conclusions seem fairly obvious—1st, there is little, if any, reinfection after a certain age; and 2nd, the tendency is for the nodules to become greatly diminished and ultimately disappear.

Intermediary Host.

As indicated in our previous communication there are several possibilities with regard to the intermediate host, which we have already shown must be present, though, as there stated, the evidence then available appeared to point to a biting insect, and especially the louse, as being the responsible agent.

Since then, experiments at that time in progress have been completed, the investigations in the Northern Territory above referred to have been made, and several other series of experiments have been carried out with the object of testing conclusively whether the infection may be brought about by direct contact, by intermediation of the soil, or by either of the lice normal to the cow, and as to whether perchance an adult worm or the larvae may leave a nodule and infect the same or other animal, thus rendering an intermediary host unnecessary.

1.—Direct Infection.

To test so far as possible whether any intermediate host be necessary, a well-formed nodule containing a living parasite was removed from a cow containing a fair number of nodules, and transplanted, under all proper conditions of asepsis, etc., to the subcutaneous tissue behind the shoulder of a calf born and reared at the Institute. Eight months later this animal was killed. The nodule was found firmly adherent to the skin, flattened and somewhat smaller in diameter than previously, and surrounded by an intimate capsule of diffuse new connective tissue, which covered it so effectually that it would have been quite overlooked had not the exact spot of the transplantation been known. The parasite was dead, and calcified in pinhead areas. Evidently, therefore, as might be expected, the parasite will not live in other than the individual host in which it has developed, and probably cannot leave the nodule once the latter is formed, and so re-infect the same animal or pass out and infect another. Also, the larvae from this living nodule had every opportunity and sufficient time to infect the new host, and to form nodules, since well-formed nodules have been found in 6-8 months old calves, so that any possibility of direct infection without the intermediation of another host is negatived, as one would have expected.

2.—Intermediation of Soil.

A quantity of earth from a cow-yard in North Queensland, wherein badly infected cows rested every night, was imported here. It was examined very carefully for any sign of larvae or adult *Onchocerca*, both before and after moistening, and after incubating for some days, with negative results.

The soil was spread evenly over a deep layer of ordinary soil in a pen in which a young locally-bred calf was placed. The pen was a warm one, and the soil was kept moist for some time after deposition. Seven months afterwards, the calf was killed and most carefully examined, and showed no trace of nodules, or of adult parasites in any part of the body, or of larvae either in the blood or in the subcutaneous areolar tissue, glands, muscles, or intermuscular tissue of the brisket; so that, at all events in this case, where all the conditions were as favourable as they could possibly be, infection by intermediation of the soil alone, is absolutely negatived.

3.—Intermediation of the Louse and Infection by Direct Contact.

A calf referred to in the previous paper, to which lice had been artificially transferred after being allowed to feed on a restricted area inoculated with numerous larvae, died some nine months after trans-

ference of the lice, but showed no sign of nodules either in the brisket or on the thigh, and no filarial parasites at all.

Two cows, each containing a considerable number of nodules, were imported to Melbourne from North Queensland, and placed in two separate pens. They were examined very carefully at different times on and after arrival for ectoparasites, with negative results. A locally-bred 6-months-old calf was placed with each cow, each calf carrying numbers of *Trichodectes scalaris* (*Haematopinus vituli* and *H. eurysternus* not being then available), these being also found later on the cows. A large number of *H. vituli* and some *H. eurysternus*, and a large quantity of their eggs were later placed on each cow, especially just over the nodule region. Large numbers of each kind of louse were examined for larvae up to and about 5 weeks after they had been placed on the cows, and at intervals later, but always with negative results. That the worms in some at least of the nodules were living was shown on arrival by excision of one of a large group lying under the skin, living larvae and eggs being numerous. The blood of the animal was examined during the operation, but no larvae could be detected. Nor were any larvae or intraparasitic parasites found in the sediment in saline fluid in which this living nodule had been kept at blood heat for some time.

Fluid aspirated after several blisterings in various ways, was examined at different times after the oedema had been produced, both on the same day and on the succeeding day, but in no case were larvae to be found.

Further numerous examinations were made of the blood at all hours of the day and night for larvae, but none could be found even after considerable quantities had been centrifuged, and a very large number of smears, both thick and thin, examined in many ways.

It is well to remember that embryos of *Onchocerca volvulus*, which forms similar subcutaneous tumours in natives in West Africa, have not yet been found in the blood of infected natives; as Brumpt (p. 457) very naturally suggests, they may in that case pass into the blood intermittently, or at certain times when the patient has not been examined.

As stated above, we (and we believe others also) have made exhaustive examinations at all hours of the day and night. There is, however, one possibility Lingard (p. 22) has found in the blood of horses in India, affected by *Filaria equina* (?) and *Filaria* sp., that comparatively few embryos are present in the blood between July and September, and may be even absent after that date. They were more numerous between December and June, being most generally present from April to September, during the hot and rainy seasons. This supports the suggestion we have already made to the effect that a seasonal periodicity may be found in *O. gibsoni*.

After an interval of 5 months, the calf in one of the pens mentioned above was killed (as the result of an accident), and showed no sign of *Onchocerca* anywhere. Two months later the other calf, which had been in habitual contact with the second cow, was killed, and also showed no evidence of either free *Onchocerca* or of nodules.

From these experiments, therefore, one may infer that neither direct contact nor apparently the intermediation of *Haematopinus vituli* or *H. eurysternus* or *Trichodectes scalaris* (though this latter would hardly be expected to act as such an agent) can act as a means of transmission of *Onchocerca gibsoni*.

This apparent failure of direct contact and of the louse to act as intermediary agencies in the spread of *O. gibsoni* is extremely interesting in the light of what has been stated earlier in this paper concerning probable introduction of the parasite in Indian cattle, since as there stated, distribution from the Indian cattle originally introduced, if they were the original hosts, could hardly have taken place other than through the agency of a flying and biting insect, though there are, as pointed out in our previous paper (p. 27) several difficulties in respect to this means of transmission.

As regards the apparent absence of embryos from the blood, and the suggested impossibility of transmission therefore by a blood-sucking insect, we may note that Brumpt (p. 457) does not hesitate to suggest in the exactly similar case of *O. volvulus* that a specific fly *Glossina palpalis* is the distributing agent.

It may be remarked that proof of the intermediation of any flying insect will be impossible without rigorous methods of experimentation, and even then will be extremely difficult. So numerous are the native animals, birds and marsupials, carrying microfilaria in their blood-streams, that only insects bred in a laboratory can be used, and they must be forms belonging to the North of Australia, since those found in the southern States are possibly impotent in this respect, while for this and other reasons, including climatic conditions, the experiments must be done in the North, with cattle bred preferably in Tasmania, so as to avoid all possible infection previous to experimentation. The practicability of, and arrangements for, experiments along these lines are now under consideration, and facilities for the same have been asked for from the Federal Government.

From the scientific point of view it is undoubtedly true that complete proof as to the intermediate host would be valuable, but from the practical point of view, we do not anticipate that the results would be of any material value. We have shown that every probability points to the intermediary host being a fly; we have also shown previously that in the northern parts of Australia all cattle are more or less affected. When one reflects, therefore, that over the greater

part of Northern Australia where worm-nodules are prevalent there are no fences, that the cattle on the average do not nearly number 1 per 100 acres (often over large areas not 1 per 1000 acres), and the impossibility of coping effectually with tick fever in such countries, where the intermediary host, the tick, is well known and cannot fly, the remoteness of practical means of prevention is evident.

In contradiction, it may be urged that certain biting insects have been eliminated from certain districts in the world; but these have been insects restricted to certain habitats such as watercourses, etc., and we have previously shown that cattle from the driest areas are often as seriously affected as cattle from wet districts, and that even areas chiefly supplied by artesian water, are not by any means immune. Nor so far as is yet determined by the evidence available at freezing works, etc., is there any natural circumstance other than latitude, which specially favours infection.

This does not mean that we suggest that scientific investigation should be discontinued, but that the stockowner and exporter can hardly expect an extermination of the parasite.

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